

The influence of high application rates of polyacrylamide on microbial metabolic potential in an agricultural soil

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Abstract

Water soluble anionic polyacrylamide (PAM) is a highly effective erosion preventing and infiltration enhancing polymer, when applied at rates of 1–10 g m⁻³ in furrow irrigation water. PAM greatly reduces sediment, nutrients, pesticides and coliform bacteria in irrigation runoff. There has been some concern about the potential for PAM accumulation to affect microbial ecology. We ran a long-term study applying massive quantities of PAM to soil and monitored its impact on soil microbial potential. In June, July and August, we measured active soil bacterial and fungal biomass and microbial diversity in soils receiving 0 (control), 2691 and 5382 kg active ingredient (ai) PAM ha⁻¹. Active bacterial biomass in soil was 20–30% greater in the control treatment than in soil treated with 2691 or 5382 kg ai PAM ha⁻¹ in June and August, but not July. Active fungal biomass in soils was 30–50% greater in the control treatment than soil treated with 2691 or 5382 kg ai PAM ha⁻¹ in June and July, but not August. Active microbial biomass in soil was 27–48% greater in the untreated control than soil treated with 2691 or 5382 kg ai PAM ha⁻¹ except in June. Whole soil fatty acid profiles showed no discernible change in the soil microbial community due to either of the PAM treatments at any sampling time. Analysis of nutritional characteristics using Biolog GN plates, however, yielded an apparent separation of the non-amended control soils from those plots receiving the high PAM application rate in June, but not in July or August. In contrast, comparisons of the three sampling times by both the fatty acid and Biolog analyses indicated that the microbial metabolic potential present in June were different from those sampled in July and August. Although PAM application to soil or irrigation water in some cases may reduce active bacterial and fungal biomass it does not seem to appreciably affect the soil microbial metabolic potential. Published by Elsevier B.V.

Keywords: Active bacterial and fungal biomass; Whole soil FAME; Biolog; Erosion

1. Introduction

Polyacrylamide (PAM) has been used in furrow irrigated agriculture for erosion control and increased infiltration (Lentz et al., 1992; Lentz and Sojka, 1994; Sojka et al., 1998a,b). PAM treatments reduce sediment loss, thus improving runoff water quality parameters, such as *ortho*-P, total-P, NO₃⁻ and biological oxygen

demand (Lentz and Sojka, 1994; Lentz et al., 1998, 2000; Entry and Sojka, 2003). Runoff sediment reduction averaged 94% and infiltration increased 15% in a series of studies conducted over 3 years (Sojka et al., 1998a,b). Additional studies documented the capacity of PAM-treatment of furrow irrigation water to reduce sediments, nutrients and pesticides in irrigation water (Agassi et al., 1995; Bahr et al., 1996; Singh et al., 1996; Sojka et al., 1998a,b; Entry and Sojka, 2003).

Fewer microorganisms in water runoff from 40 m furrows resulted from repeated application of 15–30 g of PAM directly to the soil surface in the first 1.0 m

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long \times 0.3 m wide area of the furrow (Entry et al., 2003; Sojka and Entry, 2000; Entry and Sojka, 2000). Entry and Sojka (2000) found that after water flowed over three manure sources and then PAM, PAM + $\text{Al}_2(\text{SO}_4)_3$ or PAM + CaO in furrows, total coliform bacteria, fecal coliform bacteria and fecal streptococci were reduced up to 99.9% in water flowing 1 and 27 m downstream of the treatments compared to the control treatment.

The water soluble PAMs we studied are used in erosion control and are very large anionic molecules. In industry they are used safely for a variety of food, water treatment and sensitive environmental applications (Barvenik, 1994). They should not be confused with gel forming, crosslinked PAM or evaluated with other PAM formulations, especially cationic PAMs, which have known safety concerns related to their specific chemistries (Barvenik, 1994). Environmental regulation, safety and toxicity issues related to PAM use have been extensively reviewed (Barvenik, 1994). Polyacrylamide compounds are used in many industrial processes to accelerate flocculation or at high concentrations as lubricants or sealing or suspending agents.

PAM degradation in soil was found to be approximately 10% year⁻¹ (Barvenik, 1994; Tolstikh et al., 1992). Degradation of the acrylamide monomer (AMD) is fairly rapid (Lande et al., 1979; Shanker et al., 1990; Kay-Shoemaker et al., 1998a). AMD was completely degraded within 5 days after applying 500 kg PAM kg⁻¹ garden soil (Shanker et al., 1990). Lande et al. (1979) applied 25 kg PAM kg⁻¹ soil and reported that half life of AMD in agricultural soils was 18–45 h. Enrichment cultures showed that bacteria are capable of utilizing PAM as a sole source of N, but not C (Kay-Shoemaker et al., 1998b). The effect of PAM application to water or soils has been shown to both increase and decrease soil microbial biomass (Nadler and Steinberger, 1993; Steinberger et al., 1993; Kay-Shoemaker et al., 1998a,b). There are few reports concerning the effects of PAM on the functioning of microbial metabolic potential in agricultural soils. The objective of our study was to determine the influence of very high rates of soil-applied anionic PAM on soil microbial biomass and metabolic potential.

2. Materials and methods

2.1. Study site

The study was conducted at the USDA Agricultural Research Service's Northwest Irrigation and Soils Research Laboratory in Kimberly, Idaho. The soil in

the test area was Portneuf silt loam (coarse-silty, mixed, superactive mesic Durinodic Xeric Haplocalcid), with 10–21% clay and 60–75% silt, and organic matter content of approximately 13 g kg⁻¹. Saturated paste extract electrical conductivity (EC) of this soil ranges from 0.7 to 1.3 dS m⁻¹, with exchangeable sodium percentage (ESP) of 1.4–1.7 and pH of 7.6–8.0 with a CaCO_3 equivalent of 2–8%. Slope on this site was approximately 1.5%. Soil physical and chemical parameters on these treatments are described in detail in Entry and Sojka (2003). Plots were 40 m long \times 4 m wide.

2.2. Experimental design

We took a total of 81 samples. There were three PAM treatments (5382 kg PAM ha⁻¹, 2691 kg PAM ha⁻¹ and a control \times 3 replicated plots \times 3 sampling times (June–August) \times 3 separate samples taken from each plot during each sampling period as observations (within plot replication). The experimental design was a randomized complete block with three replications (Kirk, 1982). Treatments were: 5382 kg PAM ha⁻¹, 2691 kg PAM ha⁻¹ and a control (no PAM applied) with 3 replications (plots) of each treatment. We took three samples in June, July and August, 2001 from each plot.

2.3. Crop growth, fertilizer and pesticide application

Bean, (*Phaseolus vulgaris* L.), wheat (*Triticum aestivum* L.), corn (*Zea Mays* L.) and potato (*Solanum tuberosum* L.) were grown the first year (1995) in various parts of the study area and all plots received 146 kg ha⁻¹ N and 156 kg ha⁻¹ P as well as the herbicides Ethalflurin, Cycloate, EPTC, Metribuzin and Metolachlor (Table 1). In the second and third years only bean was grown and all plots received 146 kg ha⁻¹ N and the herbicide Ethalflurin. In the fourth and fifth years only corn was grown and all plots received 168 kg ha⁻¹ N and herbicide EPTC. In the sixth year only bean was grown and all plots received 90 kg ha⁻¹ P and herbicides Ethalflurin, Cycloate, EPTC, Metribuzin and Metolachlor (Table 1).

2.4. Polyacrylamide application

The polyacrylamide copolymer used was a dry granular material having an approximate molecular weight of 12–15 Mg mole⁻¹, with an 18% negative charge density (provided by CYTEC Industries of Wayne, NJ and marketed under the trade name Superfloc 836A). PAM application involved the spread

Table 1

Crop, fertilizer and herbicide rates applied to all treatments over a 6 year period from 1995 to 2000

Year	Crop	Fertilizer (kg ha ⁻¹)		Herbicide (kg ha ⁻¹)				
		46-0-0	0-45-0	Ethalfuralin	Cycloate	EPTC	Metribuzin	Metolochlor
1	Bean, Wheat, Corn, Potato	146	156	0.58	4.27	5.86	0.34	4
2	Bean	0	0	0.58	0	0	0	0
3	Bean	168	0	0.58	0	0	0	0
4	Corn	168	0	0	0	4.34	0	0
5	Corn	168	0	0	0	4.34	0	0
6	Bean	0	90	0	0	0	0	0.49

of granular PAM on the surface of an approximately 0.1 m² area in the furrow, corresponding to the first meter of furrow below inflow spigots. Successive application amounts were 35, 35 and 15 g of material (approximately 28, 28 and 12 g of active ingredient, which is referred to as the “patch” PAM application method as described in the USDA National Resource Conservation Service conservation practice standard (NRCS, 2001; Sojka et al., 1998c)). We incorporated 897 kg PAM ha⁻¹ into the soil in the spring every year for 3 years to plots receiving 2691 kg PAM applied ha⁻¹ from 1998 to 2000 (Table 2). We incorporated 897 kg PAM ha⁻¹ into the soil in the spring every year for 6 years to plots receiving 5382 kg PAM applied ha⁻¹ from 1995 to 2000. Since the PAM contains approximately 41.5% C and 17.7% N PAM treatments received 465 kg C ha⁻¹ and 199 kg N ha⁻¹ each year while receiving 897 kg PAM (Table 2). Since Superfloc 836A polyacrylamide degrades at approximately 10% year⁻¹ in soil (Tolstikh et al., 1992), we estimated the amount of PAM present in the soil for each year (Table 2).

2.5. Water application

Water was applied as furrow irrigation from a storage pond via spigoted plastic pipe to a conventionally tilled field that had been disked to 10 cm depth in autumn and spring, then roller harrowed following incorporation of fertilizer, herbicides and PAM prior to planting. Furrows, 40 m in length were approximately 10 cm in depth, and were prepared with weighted 75 v-shaped furrow shaping tools. Furrow spacing in the various crops was 76 cm. Irrigation was on every other furrow only, in wheel-track furrows on the first irrigation, non-wheel furrows in the second irrigation and in wheel-track furrows again on the third irrigation and so forth. Per hectare sediment-loss and infiltration depth were calculated based on inflow and outflow rate measurements, sediment concentration of outflow, and spacing

between irrigated furrows, following previously published protocols (Sojka et al., 1992, 1994). Irrigation water electrical conductivity (EC) was 0.5 dS m⁻¹ with a sodium adsorption ratio (SAR) of 0.4–0.7.

2.6. Soil sampling

Three separate 2.5 cm diameter soil cores were collected from the top 30 cm of mineral soil from three different locations in each plot in June, July and August, 2001. One hundred grams mineral soil for each sampling location were collected from each plot. Soil samples were thoroughly mixed, sieved to pass a 1 mm opening, then split into 5 equal parts. Soils were analyzed for active bacterial and active fungal biomass determination and microbial community parameters as described below. Soil was stored in air and moisture-tight plastic bags at 4 °C at moisture conditions similar to those in the field. Soil was prepared for microbial analysis within 24 h of collection to minimize effects of storage on microbial activity (West et al., 1986).

2.7. Microbial analysis

Active and total bacteria and fungi numbers and biomass in soil were determined for each treatment using methods described by Ingham and Klein (1984). Active fungi were estimated by taking a 1.0 g soil sample which was diluted in 9 ml of a phosphate buffer (pH 6.0) and shaken at approximately 120 rpm for 5 min. A 1 ml aliquot was removed and stained with 1 ml of a 20 µg ml⁻¹ fluorescein diacetate (FDA) solution in a 0.2 M phosphate buffer for 3 min. One milliliter of 1.5% agar in a 0.1 M phosphate buffer (pH 9.5) was added to the FDA suspension. The sample was mixed and an aliquot placed on a microscope slide containing a cavity of known volume (Ingham and Klein, 1984). Immediately after preparation, slides were examined for FDA-stained hyphal length by

Table 2
Amount of carbon (C) and nitrogen (N) contained in polyacrylamide (PAM) treatments over a 6 year period from 1995 to 2000

Treatment	1995			1996			1997			1998			1999			2000		
	Application Rate of PAM (kg ha ⁻¹)	C (kg ha ⁻¹)	N (kg ha ⁻¹)	Application Rate of PAM (kg ha ⁻¹)	C (kg ha ⁻¹)	N (kg ha ⁻¹)	Application Rate of PAM (kg ha ⁻¹)	C (kg ha ⁻¹)	N (kg ha ⁻¹)	Application Rate of PAM (kg ha ⁻¹)	C (kg ha ⁻¹)	N (kg ha ⁻¹)	Application Rate of PAM (kg ha ⁻¹)	C (kg ha ⁻¹)	N (kg ha ⁻¹)	Application Rate of PAM (kg ha ⁻¹)	C (kg ha ⁻¹)	N (kg ha ⁻¹)
Control No PAM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2691 kg ai PAM applied ha ⁻¹	0	0	0	0	0	0	897	465	199	897	465	199	897	465	199	897	465	199
Total kg ai PAM ha ⁻¹ remaining in soil	0	0	0	0	0	0	0	0	0	897	465	199	1704	465	199	2430	465	199
5382 kg ai PAM applied ha ⁻¹	897	465	199	897	465	199	897	465	199	897	465	199	897	465	199	897	465	199
Total kg ai PAM ha ⁻¹ remaining in soil	897	465	199	1704	465	199	2430	465	199	3085	465	199	3673	465	199	4230	465	199

ai, Active ingredient, Superflo 836A polyacrylamide degrades at approximately 10% year⁻¹ in soil (Tolstikh et al., 1992). At time of sampling soils in plots receiving 2691 kg ai PAM ha⁻¹ would have 1771 kg ai PAM ha⁻¹ and soils in plots receiving 5382 kg ai PAM ha⁻¹ would have 3084 kg ai PAM ha⁻¹.

epifluorescent microscopy. Total fungal biomass was estimated by measuring the length and diameter of hyphae in 3–60 fields with phase-contrast microscopy. Three slides were evaluated from each sample and 10 fields per slide were evaluated with phase contrast microscopy for total (stained and unstained) hyphal length and three transects per field were evaluated for FDA-stained (active) hyphal length at $\times 160$ total magnification.

Iodonitrotetrazolium (INT) stain was used for counting active bacteria (Stadmatiadis et al., 1990). A 1 ml sample of initial soil suspension was diluted to a final dilution of 0.2 mg soil in 4 ml buffer. The suspension was incubated with 4 ml of filtered INT buffer for 60 min in the dark at 20 °C. Total bacteria per milliliter of water were estimated from the mean number of bacteria (fluorescent and non-fluorescent bacteria), their average diameter and length per field. Three slides were evaluated for each sample and 10 fields per slide were evaluated using epifluorescent oil-immersion microscopy to determine numbers and size of fluorescent (stained) and total bacteria (Lodge and Ingham, 1991).

Bacterial biomass were computed from the numbers of active and total bacteria and active and total fungal biomass were determined from hyphal length. Bacterial biomass was computed from the number of soil bacteria per gram of soil by assuming that the bacterial spheres were 1 μm in diameter (Jenkinson and Ladd, 1981). Active and total fungal biomass were computed by assuming average hyphal diameter to be 1 μm in diameter and then multiplying by the length of observed hyphae (Jenkinson and Ladd, 1981). A carbon to volume conversion factor of 120 $\mu\text{g C mm}^{-3}$ was used for both bacteria and fungi, assuming 1.1 g cm^{-3} wet density, 20% dry matter content and a 0.41 carbon content in the bacterium or fungus (Jenkinson and Ladd, 1981).

2.8. Fatty acid methyl ester analysis

Fatty acids were extracted from soil and transmethylated under mildly alkaline conditions as described elsewhere (Olexa et al., 2000). The resulting fatty acid methyl esters (FAMES) were analyzed with a HP 5890 gas-liquid chromatograph (Hewlett Packard, Rolling Meadows, IL, USA) equipped with a HP Ultra 2 capillary column (crosslinked phenyl methyl siloxane, 0.2 mm \times 25 m) and a flame ionization detector. The standard EUKARY chromatographic program and peak naming table (MIDI, Microbial ID, Newark, DE, USA) were used to identify FAMES by their retention times. Mixed FAME standards were obtained from MIDI to

adjust and monitor the calibration of the system. Longer chain fatty acids (>20 carbons) and those having average peak areas <500 (which was used as the minimum threshold for acceptable peak recognition) were eliminated from the statistical analyses. The former are generally associated with higher plants and animals (Kennedy, 1994), whereas eliminating the latter helped minimize the influence of intermittent small peaks on the analyses. The peak areas from the remaining FAMES were summed for a given sample and used to calculate the percent contribution of each FAME to the total peak area. Peaks identified by MIDI as “summed features” (groups of known fatty acids that are not resolved due to similar retention times) and recognized “unknowns” were treated identically to unambiguously named FAMES. The resulting data matrix for each sampling was examined by principal components analysis (PCA) based on a correlation matrix using CANOCO software (Microcomputer Power, Ithaca, NY, USA). Distances among sample FAMES for a given date of sampling were determined using Euclidean scaling of the PCA axis, whereas correlation biplot scaling was used to determine relationships among the individual samples, FAME data and treatment variables (ter Braak and Smilauer, 1998).

2.9. Biolog analysis

Gram-negative (GN) Biolog microtiter plates (Biolog, Inc., Hayward, California), each containing 95 individual carbon substrates plus a negative control, were used to determine the nutritional versatility of microbial metabolic potential from the various soil treatments. An initial 10^{-1} soil dilution was prepared by suspending 10 g of soil in 95 ml of sterile distilled water. Serial dilutions were carried out to the 10^{-3} dilution. Each well of a GN Biolog plate was inoculated

with 150 μ l of the 10^{-3} dilution for a given soil sample. Plates were incubated at 28 °C, and absorbance at 590 nm was measured at 72 h using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). The value obtained for the negative control well of each plate was subtracted from the values for the remaining 95 wells. The adjusted absorbances were analyzed by PCA as described for the FAME analysis, except that the average well color development (AWCD; Garland, 1996) was included as a covariable (Fang et al., 2001).

2.10. Statistical analysis

Microbial data were subjected to a general linear models analysis of variance (SAS Institute, 1996). Residuals were normally distributed with constant variance. Differences among treatment means were computed using the Least Square Means test ($P \leq 0.05$, $n = 9$). Differences among PAM treatments were analyzed both separately for each month. Data from each PAM treatment were not combined to determine differences among sampling dates.

3. Results

Active bacterial, fungal and microbial biomass were not consistently affected by high PAM additions. Active bacterial biomass in soil was 20–30% greater in the control treatment (not receiving PAM) than soil treated with 2691 or 5382 kg active ingredient PAM applied ha^{-1} in June and August, but not July (Table 3). There was no difference in soil active bacterial biomass between the 2691 and 5382 kg active ingredient PAM applied ha^{-1} treatments regardless of sampling time. Active fungal biomass was 30–50% greater in the control treatment than soil treated with 2691 or 5382 kg

Table 3
Active bacterial biomass, active fungal biomass and active microbial biomass after in soils treated with 2691 and 5382 kg ai PAM ha^{-1}

Month	Treatment	Active bacterial biomass (mg C g^{-1} soil)	Active fungal biomass (mg C g^{-1} soil)	Active microbial biomass (mg C g^{-1} soil)
June	Control (0 kg ai PAM applied ha^{-1})	9.04 a	10.16 a	19.21 a
	2691 kg ai PAM applied ha^{-1}	7.20 b	6.77 b	13.97 b
	5382 kg ai PAM applied ha^{-1}	7.32 b	7.24 b	14.56 ab
July	Control (0 kg ai PAM applied ha^{-1})	5.31 b	10.64 a	15.95 a
	2691 kg ai PAM applied ha^{-1}	4.86 b	6.64 b	11.51 b
	5382 kg ai PAM applied ha^{-1}	5.39 b	5.32 b	10.71 b
August	Control (0 kg ai PAM applied ha^{-1})	9.13 a	6.28 b	15.42 a
	2691 kg ai PAM applied ha^{-1}	7.20 b	6.93 b	12.54 b
	5382 kg ai PAM applied ha^{-1}	6.33 b	4.70 b	11.03 b

In each column, values followed by the same letter are not significantly different as determined by the Least Square Means Test ($P \leq 0.05$; $n = 27$).

active ingredient PAM applied ha^{-1} in June and July, but not August (Table 3). There was no difference in soil active fungal biomass between the 2691 and 5382 kg active ingredient PAM applied ha^{-1} treatments regardless of sampling time. Active microbial biomass in soil was 27–48% greater in the control treatment than soil treated with 2691 or 5382 kg active ingredient PAM applied ha^{-1} except in June with soil receiving 5382 kg active ingredient PAM applied ha^{-1} .

Analysis of nutritional characteristics using Biolog GN plates, however, yielded an apparent separation of the non-amended control soils from those plots receiving the high PAM application rate in June, but not in July or

August (Fig. 1a–c). For the June sampling, microbial communities that received the highest PAM application generally responded more strongly to a wider range of carbohydrates and fewer amino and carboxylic acids, than did those from untreated plots (data not shown). Whole soil fatty acid profiles showed no discernible change in the soil microbial community in response to any of the PAM application rates at any sampling. In contrast, comparisons of sampling times by both the fatty acid and Biolog analyses indicated that the microbial communities present at the June sampling were different from those sampled in July and August, both taxonomically and metabolically (Fig. 2a and b, respectively).

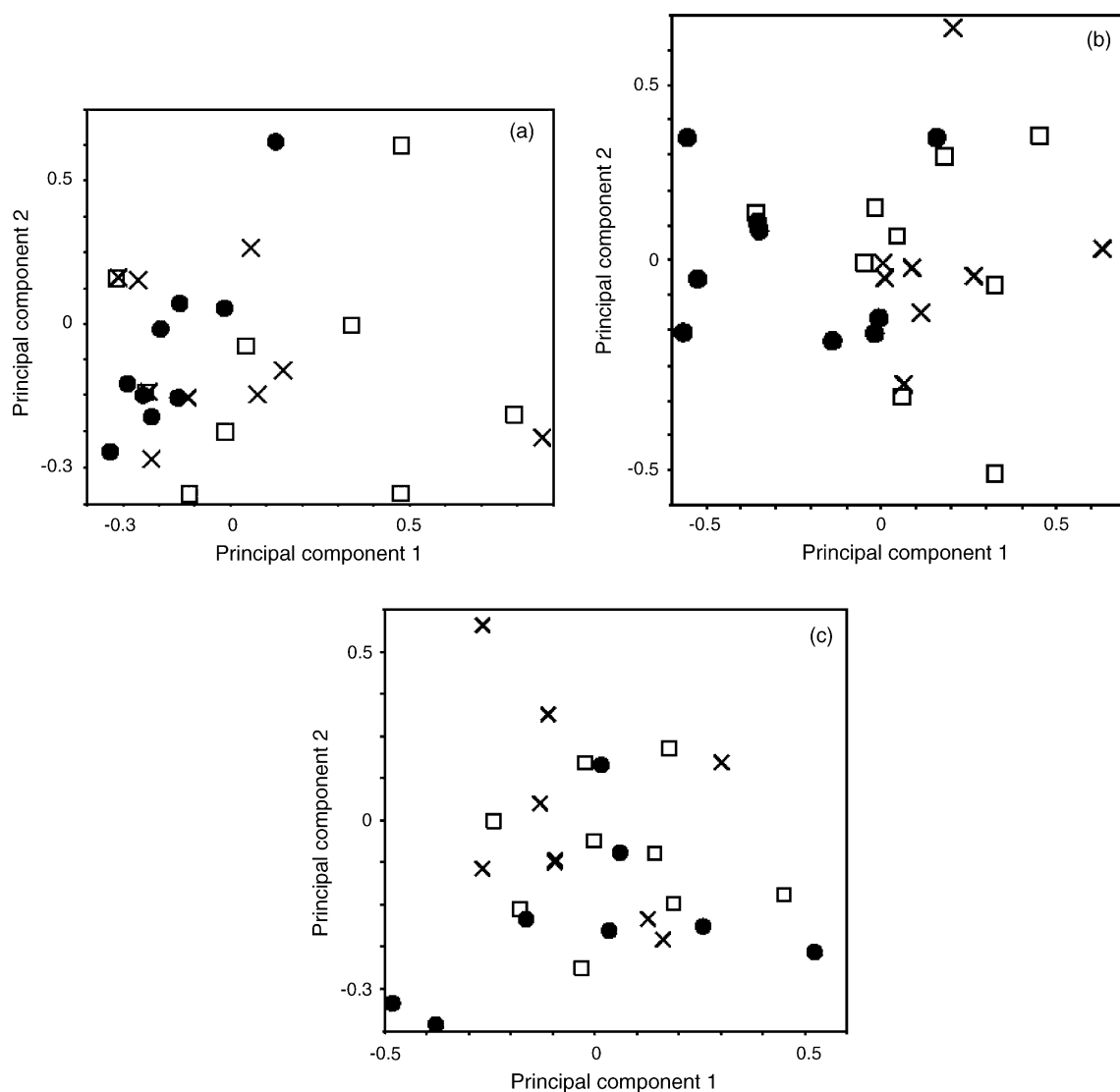


Fig. 1. Principal components analysis of substrate utilization profiles for whole-soil microbial communities exposed to various application rates of PAM and sampled in (a) June, (b) July and (c) August 2001. ((●) negative control; (×) 2691 g PAM ha^{-1} ; (□) 5362 g PAM ha^{-1}).

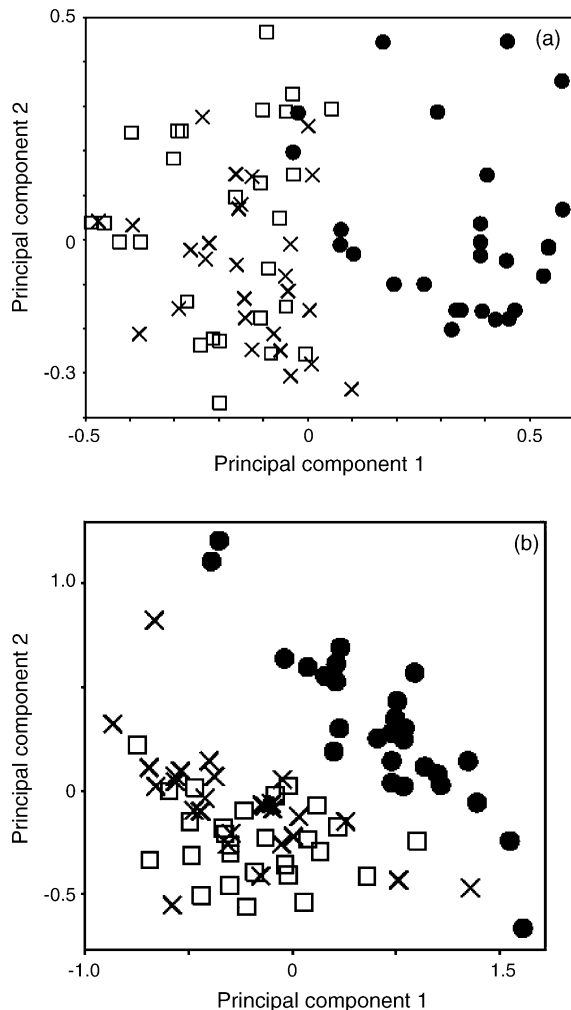


Fig. 2. Principal components analysis of (a) substrate utilization profiles and (b) fatty acid profiles for soil-whole microbial communities sampled in June (●), July (×) and August (□) 2001. Results for the PAM application rates within each type of analysis were combined for presentation.

4. Discussion

In this study, we used Superfloc A836, which is an extremely large, negatively charged molecule (Barvenik, 1994). The anionic charges on PAM use cations in the soil solution to form ion bridges between negatively charged mineral and organic surfaces, thus neutralizing charges, shrinking the electrical double layer and enabling flocculation. In this way, PAM flocculates, binds and removes soil particles, microorganisms, and nutrients in wastewater. The prevention of soil particle transport from fields to water sources keeps nutrients in the soil and out of surface waters where sediment and additional N and P contribute to algal blooms and

changes in aquatic flora and fauna (Entry and Sojka, 2003).

One might expect to find that PAM would increase microbial biomass because of the addition of the easily degradable organic material. PAM may inhibit microbial growth by binding microorganisms to soil particles or to one another, restricting their mobility and access to carbon and nutrients or reducing N fixing bacteria via nitrogen additions. Considering the extremely high rates of PAM application in this study the additional N could be affecting N mineralization and the N fixing component of the soil microbial community rather than the addition of PAM itself.

The Superfloc A836 used in this study, which has been shown to be non-toxic to most organisms (Barvenik, 1994; Seybold, 1994; Bologna et al., 1999), contains <0.05% acrylamide monomer (AMD). AMD is a neurotoxin, but PAMs below these AMD contents are considered safe under prescribed use (Barvenik, 1994). AMD is easily metabolized by microorganisms in soil and biologically active waters, with a half life in tens of hours (Lande et al., 1979; Shanker et al., 1990). Bologna et al. (1999) showed that AMD is not absorbed by plant tissues and apparently breaks down rapidly even when injected directly into living plant tissue. PAM does not revert to AMD in the natural environment upon degradation (MacWilliams, 1978). Superfloc A836 PAM degrades at rates of at least 10% year⁻¹ as a result of physical, chemical, biological and photochemical processes and reactions (Tolstikh et al., 1992). Because PAM is highly susceptible to UV degradation, its breakdown rate when applied at the soil surface for erosion control may be faster than the above-cited 10% year⁻¹ reported rate, which was for biological degradation of PAM mixed into a large soil volume.

Used at prescribed rates, anionic PAMs are environmentally safe. Negative impacts have not been documented for aquatic macrofauna, edaphic microorganisms or crop species for the anionic PAMs used for erosion control when applied at recommended concentrations and rates (Barvenik, 1994; Kay-Shoemaker et al., 1998a,b). Spackman et al. (2003) found that 10 kg PAM ha⁻¹ did not affect concentrations of total or fecal coliform bacteria in irrigation water, soil water or soils in the presence of a dense grass stand acting as a filterstrip. Nitrification of added urea appears to be somewhat accelerated (approximately 10% over 2 weeks) in PAM-treated microcosm soils (Kay-Shoemaker et al., 2000b), but no other significant impacts of PAM-application on fertilizer fate have been noted. Sorptive dynamics of the common pesticides, 2,4-D and atrazine, are not dramatically altered by PAM treatment

of field soil samples, although some slight changes in desorption and degradation rates were reported (Watwood and Kay-Shoemaker, 2000). When PAMs are introduced into waters containing sediments, humic acids or other impurities, PAM effects on biota are greatly buffered due to adsorption and deactivation associated with the suspended impurities (Goodrich et al., 1991; Buchholz, 1992). Watwood and Kay-Shoemaker (2000) found that although PAM additions to field soils correlate with discernable changes in microbial carbon utilization patterns, these effects are masked by impacts of other field variables, such as crop cover type or nutrient status.

There have been mixed, and sometimes conflicting, reports regarding the effects of PAM application on bacterial biomass levels in soils and waters (Nadler and Steinberger, 1993; Steinberger et al., 1993; Kay-Shoemaker et al., 1998a,b). Kay-Shoemaker et al. (1998a) found larger populations of culturable heterotrophic bacteria in PAM-treated soils planted to potatoes, but not in those planted to beans. These observations, along with other studies showing either increases or decreases in bacterial numbers for PAM-treated soils, indicate that these effects are likely site, season or cultural practice specific and may interact with other important variables, such as nutrient levels, crop cover type or herbicide regimes. Bacterial enrichment cultures, derived from PAM-treated field soils, were capable of growth with PAM as a sole source of N but not C, whereas AMD served as a sole source of both N and C for bacterial growth (Kay-Shoemaker et al., 1998b). A unique PAM-specific amidase was described by Kay-Shoemaker et al. (1998a,b). This enzyme, which is apparently induced by the presence of PAM in soils, breaks amide linkages found in PAM, releasing NH_4^+ which is rapidly taken up by bacteria during growth. In laboratory incubations, 20% of the N in the added PAM was removed over a period of 120 h (Kay-Shoemaker et al., 1998b). PAM-specific amidase activity has been documented in laboratory cultures, as well as in field soil samples, following exposure to PAM (Kay-Shoemaker et al., 1998b). The enzyme appears to have a broad substrate range, exhibiting activity against formamide and propionamide, but does not impact degradation rates of carbaryl, diphenamid or naphthalene acetamide in PAM-treated soils (Kay-Shoemaker et al., 2000a). Intracellular and extracellular activity have been noted, and production and secretion of the enzyme appears to be dependent on C availability, as cells cannot derive C directly from PAM. Elevated soil N concentrations have been shown to reduce microbial biomass and mineralization of cellulose, lignin and

herbicide (Entry, 1999, 2000; Entry et al., 1993; Entry and Backman, 1995). When large amounts of PAM are applied to soil, additional N contained in PAM may reduce microbial biomass.

The most important finding of this work is that in spite large accumulative additions of PAM over 3–6 year periods, there was little notable effect on soil microbial biomass and metabolic potential as measured by Gram-negative Biolog microtiter plates or whole soil FAME. Furthermore, effects on the size of soil microbial populations, though measurable, were inconsistent and small considering the massive amounts of PAM added. Occasional questions have arisen during the past decade as to whether the use of anionic PAM for soil structure enhancement or erosion control could have negative effects on soil microbial populations or diversity. Our results from these massive PAM application rates and prolonged exposure times lead us to conclude that these consequences are highly unlikely. To the extent we saw soil microbial biomass shifts, interpretation in light of earlier literature suggests it could be due to N enrichment of the PAM addition rather than the PAM polymer chemistry. The possible small shifts in soil microbial biomass were judged to be insignificant when weighed against the significant erosion prevention and runoff water quality protection afforded by the use of 10–15 kg PAM ha^{-1} per season for erosion control, compared to the 2691 and 5382 kg active ingredient PAM ha^{-1} application rates used in this study. In order to achieve the concentrations of PAM added to soil in this study, assuming no PAM degradation, land managers would need to apply 15 kg active ingredient PAM ha^{-1} for 180 years to reach the 2691 active ingredient PAM applied ha^{-1} and 360 years to reach the application rate of 5382 kg active ingredient PAM applied ha^{-1} .

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